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14. ABSTRACT Purpose: Lyophilized plasma (LP) is a light weight powder that remains stable for a prolonged period of time and in a broad range of temperatures. The powder is rapidly reconstituted into a product that preserves coagulation factor activity and suppresses harmful inflammation. The purpose of this project was to demonstrate that LP could be optimized by minimizing the reconstitution fluid and improving the constituents. Scope: Specific aim 1 focused on determining the minimum amount of fluid required to reconstitute a safe and effective product. Specific aim 2 evaluate four fluid options for reconstitution. Specific aim 3 evaluated the concentration of ascorbic acid used to buffer the LP and potentially reduce the amount of inflammation due to injury. Finally, specific aim 4 involved development of a survival model that utilized the final optimized LP product. Findings: LP can be safely reconstituted with 50% of the original volume with sterile water being the best fluid for reconstitution. Ascorbic acid can safely be used to buffer the LP, and the concentration does not make a difference.					
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INTRODUCTION:

Trauma is the leading cause of death among civilians between the ages of 1 and 44. Military casualties are more likely than civilian casualties to die from hemorrhage. Exsanguination is the leading cause of preventable death after combat-related injury. There is increasing evidence from both military and civilian sources that resuscitation with high ratios of plasma to packed red blood cells results in improved survival in patients who undergo massive transfusions. FFP is not available in the field or far forward settings. Even in the hospital, FFP cannot be immediately available in sufficient quantities to support a high ratio approach to massive transfusion. These problems are magnified in mass casualty situations. Our collaborators have developed a method of lyophilizing, or freeze-drying, plasma into a powder form which is reconstituted in water containing vitamin C to normalize the pH. We are currently reconstituting the plasma to its original volume and we have measured coagulation factor activities in the reconstituted plasma. We have determined that lyophilization and reconstitution produces, on average, factor activity retention of 86%. This is superior to standard FFP which has retained factor activities ranging from 60% to 70%. We have also shown in a severe multiple injury swine model that lyophilized plasma (LP) is equivalent to fresh frozen plasma with respect to physiology, coagulopathy and survival. We did not find differences between swine treated with LP and FFP in coagulation profiles, serum clotting factor levels, lactate, post-injury blood loss, survival, or hemodynamic parameters. The lyophilized plasma also suppressed dysfunctional inflammation compared to fresh frozen plasma, presumably due to the vitamin C which possesses anti-oxidant properties possibly inhibiting oxidative stress.

BODY:

Specific Aim 1 Materials – To determine the minimum amount of fluid necessary to successfully reconstitute lyophilized plasma without reducing its efficacy in a swine multiple injury model of hemorrhagic shock. Animals will be randomized to receive full volume of plasma or reduced volume of plasma.

This model was developed at Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy that lyophilized plasma reconstituted with a smaller volume will result in similar or better results than fully reconstituted lyophilized plasma.

Blood Collection for Plasma Preparation

All experimental procedures were done in accordance with the guidelines of the Institutional Animal Care and Use Committee at Oregon Health & Science University. Blood products used in this study were obtained from juvenile Female Yorkshire crossbred swine. Using sterile precautions, a cervical cut down was performed and the carotid artery was cannulated with an 8F introducer (Argon Medical Devices, Athens, Texas). Animals were exsanguinated and blood was collected into citrated blood donation bags (Teruflex; Terumo Medical Corp, Tokyo, Japan). Whole blood was centrifuged at 5000g for 9 minutes at 4°C. The plasma was then removed using a plasma extractor (Baxter Healthcare, Deerfield, Illinois). Plasma was stored at -20°C for transport to a laboratory (HemCon Medical Technologies Inc, Portland, Oregon) for lyophilization. Pooled sterile LP was returned to us and stored at room temperature for up to 2 months.

Plasma Clotting Factor Level Measurements:

Samples of the plasma were analyzed for fibrinogen, coagulation factors II, V, VII, VIII, IX, X, XI, XII, and XIII using a coagulation system machine (BCS; Dade Behring Inc, Marburg, Germany) at the time of plasma collection and following reconstitution of each study fluid. Blood from each study animal was also collected for coagulation factor analysis at all time points during the study.

Animal Model:

Juvenile, female Yorkshire crossbred swine were subjected to a well-validated swine model of severe injury and hemorrhagic shock for each specific aim (Figure 1). Animals were delivered 7 to 10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. A single vendor was utilized to eliminate potential differences in animal strain.

Anesthesia

On the day of the experiment animals were given an induction agent consisting of 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) given intramuscularly. Animals were placed in the supine position. Orotracheal intubation was performed and a 7.5mm internal diameter cuffed

endotracheal tube was placed. The endotracheal tube was connected to the anesthesia machine with 1-3% isoflurane for anesthetic maintenance in 50% oxygen. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. An esophageal stethoscope, gastric tube and thermometer were inserted. An EKG monitor was secured and continuous monitoring started. Throughout the study, anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures

After swine were anesthetized a left cervical cutdown was performed and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for the controlled hemorrhage and blood sampling throughout the experiment while the venous line was used for administration of bolus resuscitation fluids and TXA. Finally, a proximal femoral cutdown was performed and the artery was cannulated for continuous blood pressure monitoring. Mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG) and additional coagulation factors. In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury Phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. A controlled hemorrhage was then initiated to remove 60% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During hemorrhage if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP > 25 mm/Hg. The animal was also cooled to 33 +/- 0.4°C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, factors and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage to induce acidosis and coagulopathy. This reflects current civilian pre-hospital resuscitative practices.

Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed; during which a baseline MAP was recorded and pre-weighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp. The liver injury was designed to

provide a second stressor after initial injury and also to create a standardized injury that had the potential to re-bleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury.

Randomization

Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen. Following the uncontrolled hemorrhage period, the liver was packed tightly with laparotomy sponges. Swine were randomized to receive either LP reconstituted to 50% (50%LP, n=10) or 100% (100%LP, n=10) of the original plasma volume. Study fluid resuscitation was initiated at the time of liver packing. The animal was also re-warmed to 37°C and the abdomen closed with towel clips.

Follow-up

Animals were monitored for 4 hours post injury or to death. Labs were collected at 1, 2, 3 and 4 hours. If the MAP fell below 15 mmHg it was denoted as death and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. A necropsy was performed and the liver injury graded using the American Association for the Surgery of Trauma (AAST) liver injury grading system to ensure adequacy and similarity of injuries between groups.

Heart (HR) rate and blood pressure (MAP) were continuously recorded throughout the study. Blood loss following liver injury was carefully recorded with the use of pre-weighed laparotomy sponges and pre-weighed suction canisters.

Statistical Analysis

Variables were assessed for normal distribution. Normally distributed data were reported as means with standard deviations. Comparisons between groups at various time points were analyzed by independent t-tests when the data were normally distributed. Paired-samples t-tests were used to compare same-group samples across various time points. Significance was denoted at $p < 0.05$. Data were analyzed utilizing SPSS statistical software, version 19.0 (IBM Corp. Released 2010. Armonk, NY).

Results

In vitro

To ascertain the minimal volume sufficient for reconstitution of LP, reconstitution was done using decreasing volumes of sterile water with ascorbic acid as buffer. We decreased the volume in stepwise fashion using 10% volume increments. In vitro analysis of LP was successfully reconstituted using 30% of the original plasma volume. However, when intravenously administering LP reconstituted to 30% and 40% of the original plasma volumes to animals during model development, the fluids were not well tolerated. These animals died prior to or shortly after completion of the LP fluid infusions. The 50%LP solution was well tolerated in all animals.

The 50%LP solution had significantly higher concentrations of electrolytes and albumin (Table 1). Additionally, 50%LP was hyperosmolar with significantly higher osmolarity

compared to the 100%LP solution. The pH of the two study fluids following reconstitution using sterile water with ascorbic acid as buffer was not different (Table 1).

Regarding coagulation factor activity, there was no significant difference between pre-lyophilized plasma (FFP) and 100%LP (Figure 2). We found significantly increased coagulation factor activity (Fibrinogen, II, V, VII, VIII, IX, X, XI, and XII) per unit volume in the 50%LP fluid compared to FFP and 100%LP (all $p < 0.03$). TEG parameters were not different at any time point between the fluid study groups (R time, K, α – angle, or MA, $p > 0.17$).

In vivo

All 20 swine randomized to receive either 50%LP (n=10) or 100%LP (n=10) study fluid survived the study period. At baseline, animals were similar between study fluid groups (Table 2). Serum lactate increased in both study groups following femur fracture and controlled hemorrhage (Figure 3). There was no significant difference in changes in serum lactate between study groups throughout the study at any time point (Figure 4). There was no statistically significant difference in blood loss at any time point following liver injury and total blood loss at the end of the study period between the study groups (Figure 4). There was also no significant difference in hemodynamic parameters (HR and MAP) between study groups (Figure 5). No difference in hematocrit (Hct) was found between study groups at any time point (Figure 5). Analysis of the coagulation and inflammatory markers are currently ongoing for the in vivo aspect of the project.

Specific Aim 2 – To determine the optimal fluid at 50% normal volume for reconstituting lyophilized plasma without reducing its efficacy in a multiple injury model of hemorrhagic shock in swine.

Specific Aim 2 Methods –

This model was developed at Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee. The same injury model in Specific Aim 1 was used for Specific Aim 2.

Randomization

Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen. Following the uncontrolled hemorrhage period, the liver was packed tightly with laparotomy sponges. Swine were randomized to receive one of four different low-volume LP solutions reconstituted with 1) sterile water (LP-SW), 2) normal saline (LP-NS), 3) lactated Ringer's (LP-LR), or 4) Hextend[®] (LP-Hx). Study fluid resuscitation was initiated at the time of liver packing. The animal was also re-warmed to 37°C, and the abdomen closed with towel clips.

Follow-up

Animals were monitored for 4 hours post injury or to death. Blood samples were collected at 1, 2, 3, and 4 hours. A MAP below 15 mmHg signified death, and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rt-PCR analysis. Tissue was stored in RNA later. A necropsy was performed and the liver injury graded using the American Association for the Surgery of Trauma (AAST) liver injury grading system to ensure adequacy and similarity of injuries between groups.

Heart (HR) rate and MAP were continuously recorded throughout the study. Blood loss following liver injury was carefully recorded with the use of pre-weighed laparotomy sponges and pre-weighed suction canisters.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Point-of-care laboratory values included TEG, Hct, lactate, platelets, ABG, and electrolytes. Additional assays completed after the experiment include INR, PTT, fibrinogen, IL-6, IL-8, IL-10, and TNF- α .

Statistical Analysis

Variables were assessed for normal distribution. Normally distributed data were reported as means with standard deviations. Comparisons between groups at various time points were analyzed by independent t-tests when the data were normally distributed. Paired-samples t-tests were used to compare same-group samples across various time points. Significance was denoted at $p < 0.05$. Data were analyzed utilizing SPSS statistical software, version 19.0 (IBM Corp. Released 2010. Armonk, NY).

Results

Of the 40 swine randomized in the study, one animal died shortly after infusion of the LP-Hx solution was completed. At baseline, animals were similar in weight, hematocrit, lactate, base excess, and pH ($p > 0.05$, all comparisons).

There were no differences between groups in hemodynamic parameters (HR and MAP, Figure 6). There were significant differences in total blood loss between the LP-LR group and the LR-NS and LR-Hx groups ($p < 0.05$, Figure 7). Additionally, the LR-SW group lost the least amount of blood over the study and was significantly lower compared to the LP-Hx group ($p < 0.05$).

Prior to initiation of the LP study fluids, the changes in hematocrit were not different between study groups at any time point, ($p > 0.05$, Figure 8). Following resuscitation, the hematocrit of the LP-Hx group was significantly lower than the LP-SW group at the 1-hour, 2-hour, 3-hour, and 4-hour time points ($p < 0.05$, Figure 8). Serum lactate increased in all four fluid groups. There was a trend towards higher serum lactate levels in the LP-LR group at 1-hour; however, there was no statistically significant difference between groups at any time point ($p > 0.05$, Figure 8). Prior to resuscitation with the LP study solutions, all animals became similarly acidotic following NS resuscitation following femur fracture and controlled hemorrhage. Following LP study solution resuscitation the serum pH increased in all groups and was not different between LP fluid groups at any time point thereafter ($p > 0.05$, Figure 8).

The INR was increased in all animals following femur fracture, controlled hemorrhage, and NS resuscitation (Figure 9). There was correction of the INR in all four study groups following resuscitation with the LP solutions following liver injury. However, the INR of the LP-Hx group remained significantly higher than the LP-NS, LP-LR, and LP-SW groups at 4-hours, ($p < 0.05$).

There were no differences in the serum concentrations of IL-10 and TNF- α ($p > 0.05$). IL-6 serum concentrations, however, were significantly lower in the LP-SW group compared to the LP-NS Group ($p < 0.05$, Figure 10).

Specific Aim 3 Materials – To determine if increasing the concentration of vitamin C in lyophilized plasma will increase its antioxidant effect suggesting the potential to reduce acute respiratory distress syndrome and multiple organ failure in combat casualties.

This model was developed at Oregon Health & Science University (OHSU) and approved by the Institutional Animal Care and Use Committee. The same injury model in Specific Aim 1 was used in Specific Aim 3.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy of lyophilized plasma reconstituted with sterile water at 50% volume with three concentrations of ascorbic acid: low (7.5mM), medium (15.0mM), and high (22.5mM) plus a hydrochloric acid (12.0mM) control.

Randomization

Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen. Following the uncontrolled hemorrhage period, the liver was packed tightly with laparotomy sponges. Swine were randomized to receive one of four acid concentrations reconstituted with low volume sterile water. The four acid concentrations employed were 7.5mM (low AA), 15.0mM (medium AA) or 22.5mM (high AA) and 12.0mM of HCL served as the control. Study fluid resuscitation was initiated at the time of liver packing. The animal was also re-warmed to 37°C, and the abdomen closed with towel clips.

Follow-up

Animals were monitored for 4 hours post injury or to death. Blood samples were collected at 1, 2, 3, and 4 hours. A MAP below 15 mmHg signified death, and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rt-PCR analysis. Tissue was stored in RNA later. A necropsy was performed and the liver injury graded using the American Association for the Surgery of Trauma (AAST) liver injury grading system to ensure adequacy and similarity of injuries between groups.

Heart (HR) rate and MAP were continuously recorded throughout the study. Blood loss following liver injury was carefully recorded with the use of pre-weighed laparotomy sponges and pre-weighed suction canisters.

Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Point-of-care laboratory values included TEG, Hct, lactate, ABG, and electrolytes. Blood samples were sent to a central core IDEXX Laboratory for CBC analysis. Additional assays completed after the experiment include INR, PTT, fibrinogen, IL-6, IL-8, IL-10, and TNF- α .

Statistical Analysis

Variables were assessed for normal distribution. Normally distributed data were reported as means with standard deviations. Comparisons between groups at various time points were analyzed by independent t-tests when the data were normally distributed. Paired-samples t-tests were used to compare same-group samples across various time points. Significance was denoted at $p < 0.05$. Data were analyzed utilizing SPSS statistical software (IBM Corp. Released 2010. Armonk, NY).

Results

Forty-six animals were included in this study with 10 animals per study acid group and 6 operative control shams (OCS). Five major blood collection time points were assessed: baseline (BL), post-liver injury at 1- (1HR), 2- (2HR), 3- (3HR) and 4- (4HR) hours between OCS and treatment groups as well as comparisons between HCL and AA groups (Tables 2 - 6). All data is presented as medians with interquartile ranges (IQR).

Operative Control Shams versus Treatment Acid Groups

All animals were similar in weight, HR, MAP, hematocrit, white blood cell count, base deficit (BD), blood pH, ACT, and TEG coagulation parameters at baseline ($p > 0.05$; all comparisons). As might be expected differences were seen between the OCS and treatment groups in MAP, HR, CBC, and chemistry and coagulation variables as outlined in Tables 2 – 6.

Hydrochloric Acid Control versus Ascorbic Acid Groups

All animals receiving LP transfusion survived the four hour observation period following liver injury. To control for the effects of hemodilution, a similar amount of NS resuscitation fluid was given to all treatment groups prior to liver injury ($p > 0.05$). The median volume of LP transfused 800 (798, 801) milliliters, pH of LP prior to transfusion 7.02 (7.00, 7.04) and time to reconstitute LP 80 (70, 95) seconds were similar between all groups ($p > 0.05$). Urine output was measured hourly following liver injury, and no differences were seen between groups ($p > 0.05$). Hemodynamically, no differences were seen between groups for 30-second blood loss, post-injury blood loss, total blood loss or HR ($p > 0.05$; all comparisons). MAP was similar between all groups until four hours, where MAP was elevated in the medium AA group versus HCL control ($p < 0.05$). All treatment animals experienced a similar degree of shock, hypoperfusion, and complete blood count versus HCL control (Table 1). Regarding coagulation, the time to clot

initiation was prolonged in the low and medium AA groups at one hour ($p<0.05$; both), and the low AA group continued to demonstrate prolonged clot initiation at four hours versus control ($p<0.05$). Overall, there were no differences in the rate of clot formation (TEG α), $p>0.05$; all comparisons. The CI was significantly decreased only in the low AA group at 1- and 4-hours post-liver injury versus HCL control ($p<0.05$; both). Taken together, all three ascorbic acid groups had pro-coagulant function within the normal range from 2 to 4 hours following liver injury.

KEY RESEARCH ACCOMPLISHMENTS

Specific Aim 1

1. Less than 50% reconstitution not well tolerated but soluble
2. 50%LP displayed significantly greater activity of all coagulation factors
3. No statistically significant differences between the groups at baseline
4. No statistically hemodynamic or laboratory difference between animals post infusion

Specific Aim 2

1. LP-SW showed less coagulopathic changes following injury compared to other groups.
2. Lower IL-6 concentrations at end of study in the LP-SW group.
3. Less total blood loss in the LP-SW group.
4. No statistically significant differences between the groups at baseline.
5. No statistically hemodynamic differences between animals following resuscitation.

Specific Aim 3

1. No differences in mortality between groups.
2. The different concentrations of ascorbic acid were physiologically well tolerated.
3. Blood loss was not different between groups.
4. Pro-coagulant coagulation properties were not decreased with increasing ascorbic acid concentrations.

REPORTABLE OUTCOMES

Specific Aim 1

1. 39th Annual Critical Care Symposium – Oregon Chapter of Society of Critical Care Medicine, November 12 – 13, 2012 in Vancouver, WA. . “Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma”.
Winner of Best Trainee Competition
2. Eastern Association for the Surgery of Trauma (EAST) 26th Annual Scientific Assembly, November 15 – 19, 2012 in Scottsdale, AZ. “Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma”.
Winner of Raymond H. Alexander, MD Resident Paper Competition
3. Oregon / Washington American College of Surgeons Committee on Trauma, Region X Conference, December 1, 2012 in Centralia, WA. “Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma”.
Winner of Best Basic Science Paper

4. American College of Surgeons – Oregon / Washington Regional Conference, June 16, 2012 in Sunriver, OR. “Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma”.
Winner of Baker-Mosely Award for Excellence in Resident Laboratory Research
5. Oregon Health & Science University Research Week May 9, 2012 in Portland, OR. “Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma”.

Specific Aim 2

American Association of Surgical Trauma 72nd Annual Meeting, September 20, 2013 in San Francisco California. “Comparison of the hemostatic efficacy of low volume lyophilized plasma reconstituted using sterile water, lactated Ringer’s, normal saline, and Hextend® solutions”.

Specific Aim 3

High, Medium and Low Ascorbic Acid Concentrations in Reconstituted Lyophilized Plasma Demonstrate Comparable Physiologic Responses Following Polytraumatic Injury.

Presented at:

1. 2014 Portland Surgical Society Meeting, Portland, OR
2. 2014 Oregon Washington American College of Surgeons Meeting, Sunriver, OR
3. 2015 Eastern Association for the Surgery of Trauma, Lake Buena Vista, FL

Bibliography Of Published Work Over The Course Of The Grant

1. Lee TH, Watson K, Fabricant L, Barton J, Differding J, Kremenevskiy I, Sands C, Wiles C, Watters JM, Schreiber MA. Hyperosmolar Reconstituted Lyophilized Plasma is an Effective Low-volume Hemostatic Resuscitation Fluid for Trauma. *Journal of Trauma and Acute Care Surgery*. 2013;75:369-375.
2. Lee TH, McCully SP, McCully BH, Sands C, Hampton DA, Louis SG, Rick B, Anderson N, Differding J, Schreiber MA. Comparison of the Hemostatic Efficacy of Low-volume Lyophilized Plasma Reconstituted Using Sterile Water, Lactated Ringer’s, Normal Saline and Hextend Solutions. *Journal of Trauma and Acute Care Surgery*. 2014;76:264-271.
3. McCully SP, Lee TH, McCully BH, Sands CL, Rick EA, Dean RK, Anderson NW, Hampton DA, Louis SG, Differding JA, Schreiber MA. Reconstitution fluid type does not affect pulmonary inflammation or DNA damage following infusion of lyophilized plasma. *J Trauma Acute Care Surg*. 2015 Feb;78(2):231-7; discussion 237-9.
4. McCully SP, Martin DT, Cook MR, Gordon NT, McCully BH, Lee TH, Dean RK, Rick EA, Moren AM, Fair KA, Undarraga VJ, Watson KM, Anderson NW, Schreiber MA. Effect of Ascorbic Acid Concentrations on Hemodynamics and Inflammation Following Lyophilized Plasma Transfusion. *Journal of Trauma and Acute Care Surgery*. 2015;79:30-38.

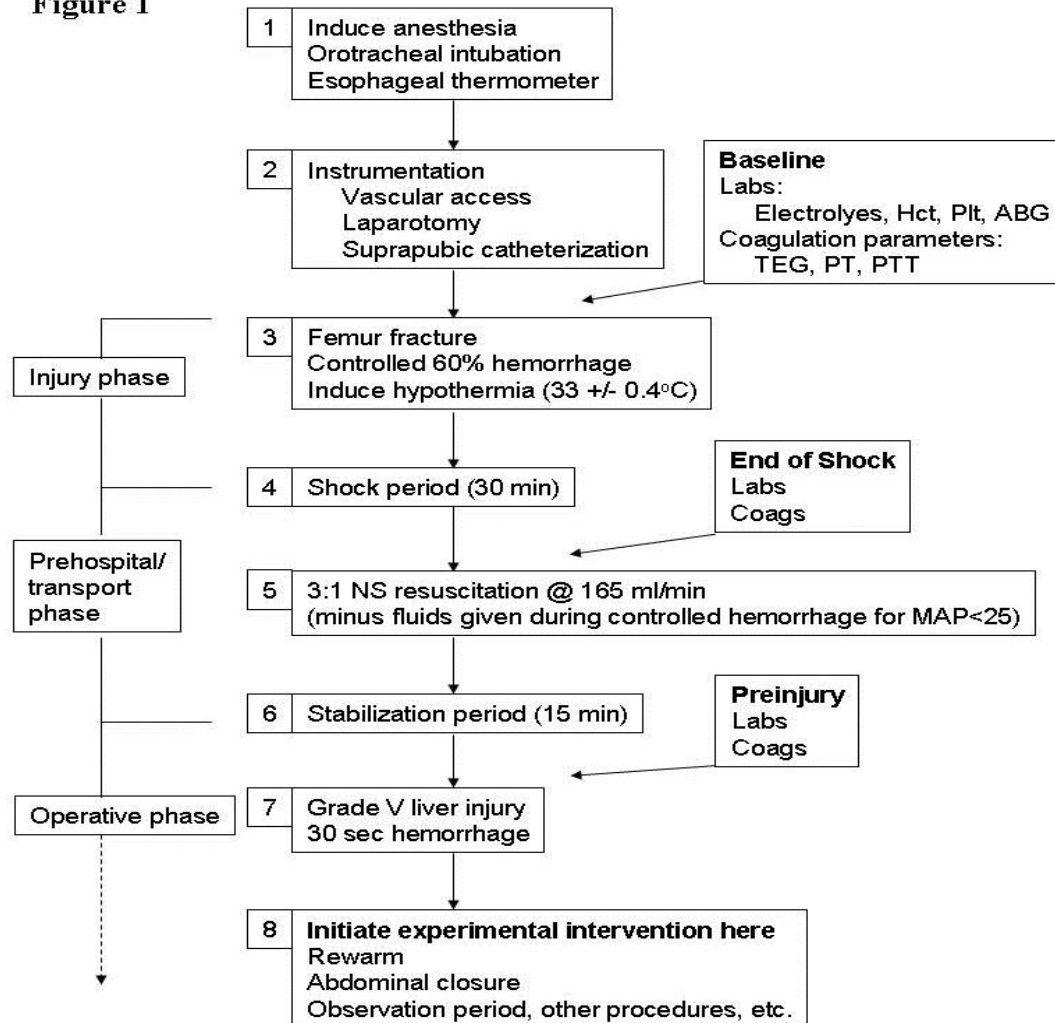
REFERENCES

- Mattox KL, Moore EE, Feliciano DV. *Trauma, 7th edition*. McGraw-Hill Professional; 2012.
- Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: A reassessment. *Journal of Trauma-Injury Infection & Critical Care*. 1995;38(2):185-193.
- Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: A review of mechanisms. *The Journal of Trauma: Injury, Infection, and Critical Care*. 2008;65(4):748 <last_page> 754.
- Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *J Trauma*. 2007;63(4):805-813.
- Sperry JL, Ochoa JB, Gunn SR, et al. An FFP:PRBC transfusion ratio $\geq 1:1.5$ is associated with a lower risk of mortality after massive transfusion. *Journal of Trauma-Injury Infection & Critical Care*. 2008;65(5):986-993.
- Holcomb JB, Wade CE, Michalek JE, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg*. 2008;248(3):447-458.
- Zink KA, Sambasivan CN, Holcomb JB, Chisholm G, Schreiber MA. A high ratio of plasma and platelets to packed red blood cells in the first 6 hours of massive transfusion improves outcomes in a large multicenter study. *The American Journal of Surgery*. 2009;197(5):565-570.
- Murad MH, Stubbs JR, Gandhi MJ, et al. The effect of plasma transfusion on morbidity and mortality: A systematic review and meta-analysis. *Transfusion*. 2010;50(6):1370-1383.
- Holcomb JB, del Junco DJ, Fox EE, et al. The prospective, observational, multicenter, major trauma transfusion (PROMMTT) study: Comparative effectiveness of a time-varying treatment with competing risks. *JAMA Surg*. 2013;148(2):127-136.
- Johansson PI, Stensballe J. Hemostatic resuscitation for massive bleeding: The paradigm of plasma and platelets--a review of the current literature. *Transfusion*. 2010;50(3):701-710.
- Curry NS, Davenport RA, Hunt BJ, Stanworth SJ. Transfusion strategies for traumatic coagulopathy. *Blood Rev*. 2012;26(5):223-232.
- Bux J, Dickhorner D, Scheel E. Quality of freeze-dried (lyophilized) quarantined single-donor plasma. *Transfusion*. 2013.
- Spoerke N, Zink K, Cho SD, et al. Lyophilized plasma for resuscitation in a swine model of severe injury. *Arch Surg*. 2009;144(9):829-834.
- Lee TH, Van PY, Spoerke NJ, et al. The use of lyophilized plasma in a severe multi-injury pig model. *Transfusion*. 2013;53 Suppl 1:72S-79S.

Lee TH, McCully SP, McCully BH, et al. Comparison of the hemostatic efficacy of low-volume lyophilized plasma reconstituted using sterile water, lactated ringer's, normal saline, and hextend solutions. *J Trauma Acute Care Surg.* 2014;76(2):264-1; discussion 271-2.

Appendix A – Animal model study design

Figure 1



Appendix B – Specific Aim 1 Tables and Figures

	50%LP (n=8)	100%LP (n=8)	<i>p</i>
Na (mmol/L)	297 ± 48	171 ± 22	< 0.05
K (mmol/L)	9.2 ± 3.1	4.7 ± 1.3	0.002
Cl (mmol/L)	139 ± 30	80 ± 14	< 0.05
Ca (mmol/L)	11.0 ± 2.5	6.5 ± 0.9	< 0.05
Alb (mmol/L)	2.0 ± 0.3	1.0 ± 0.2	< 0.05
Osmolarity (osmol/L)	621 ± 118	329 ± 44	< 0.05
pH	7.11 ± 0.11	7.18 ± 0.08	0.18

Table 1. Study fluid analysis (50%LP and 100%LP). All values expressed as group mean ± SD. (Na: sodium, K: potassium, Cl: chloride, Ca: calcium, Alb; albumin)

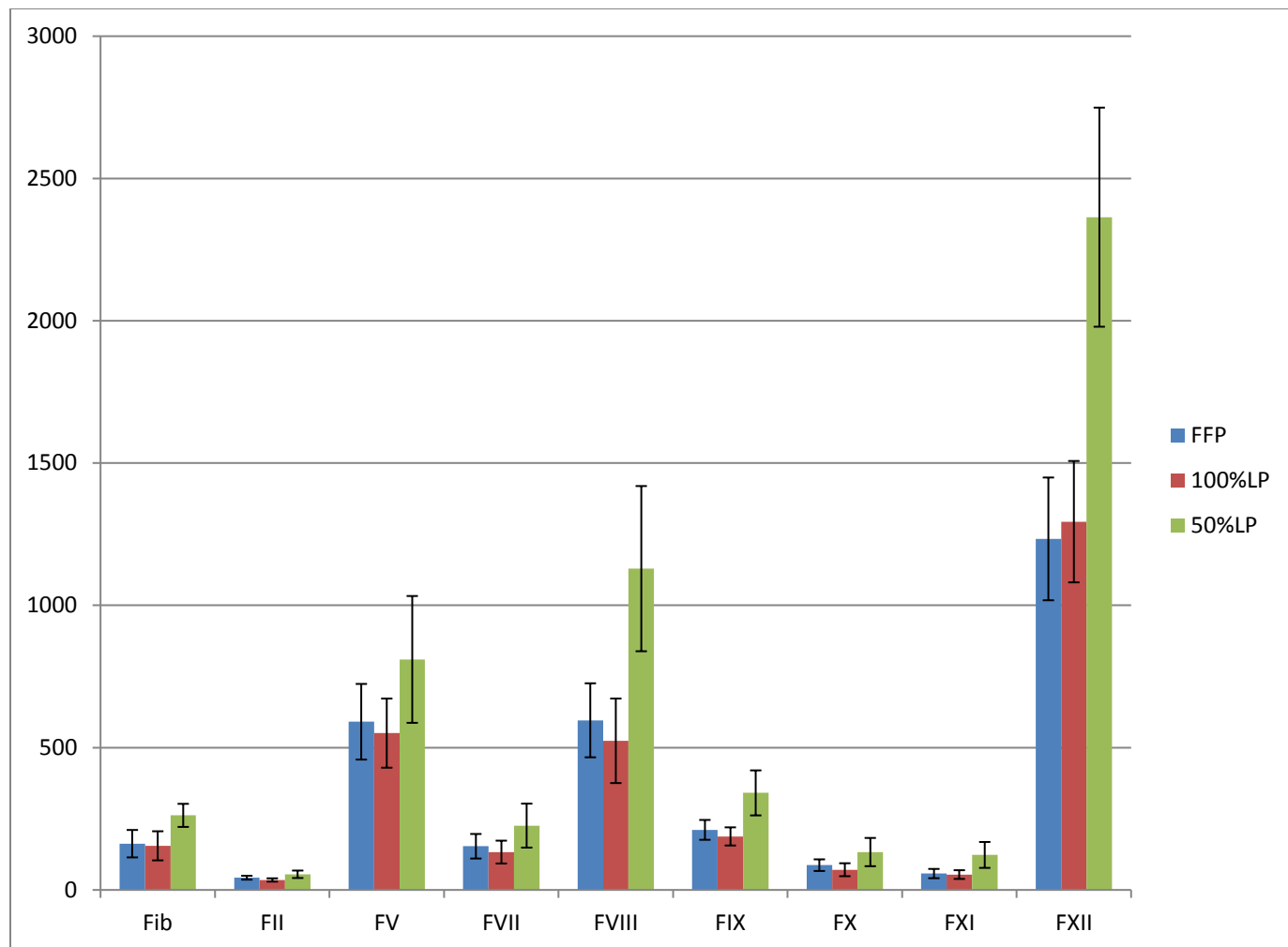


Figure 2. 50%LP has significantly greater activity of all coagulation factors compared to FFP and 100%LP, all $p < 0.03$. All values expressed as mean \pm SD. (Fib: fibrinogen, FII: Factor II, FV: Factor V, FVII: Factor VII, FVIII: Factor VIII, FIX: Factor IX, FX: Factor X, FXI: Factor XI, FXII: Factor XII).

	50%LP (n=10)	100%LP (n=10)	<i>p</i>
Weight (kg)	32.8 ± 1.3	32.3 ± 2.0	NS
Hct (%)	28.7 ± 1.1	29.2 ± 2.1	NS
Lactate (mmol/L)	1.4 ± 0.3	1.5 ± 0.5	NS
Base excess (mmol/L)	12.9 ± 2.2	11.8 ± 1.3	NS

Table 2. Baseline characteristics of animals in each study fluid group (50%LP and 100%LP). All values expressed as mean ± SD, NS: No significant difference, all $p > 0.05$; Hct: hematocrit.

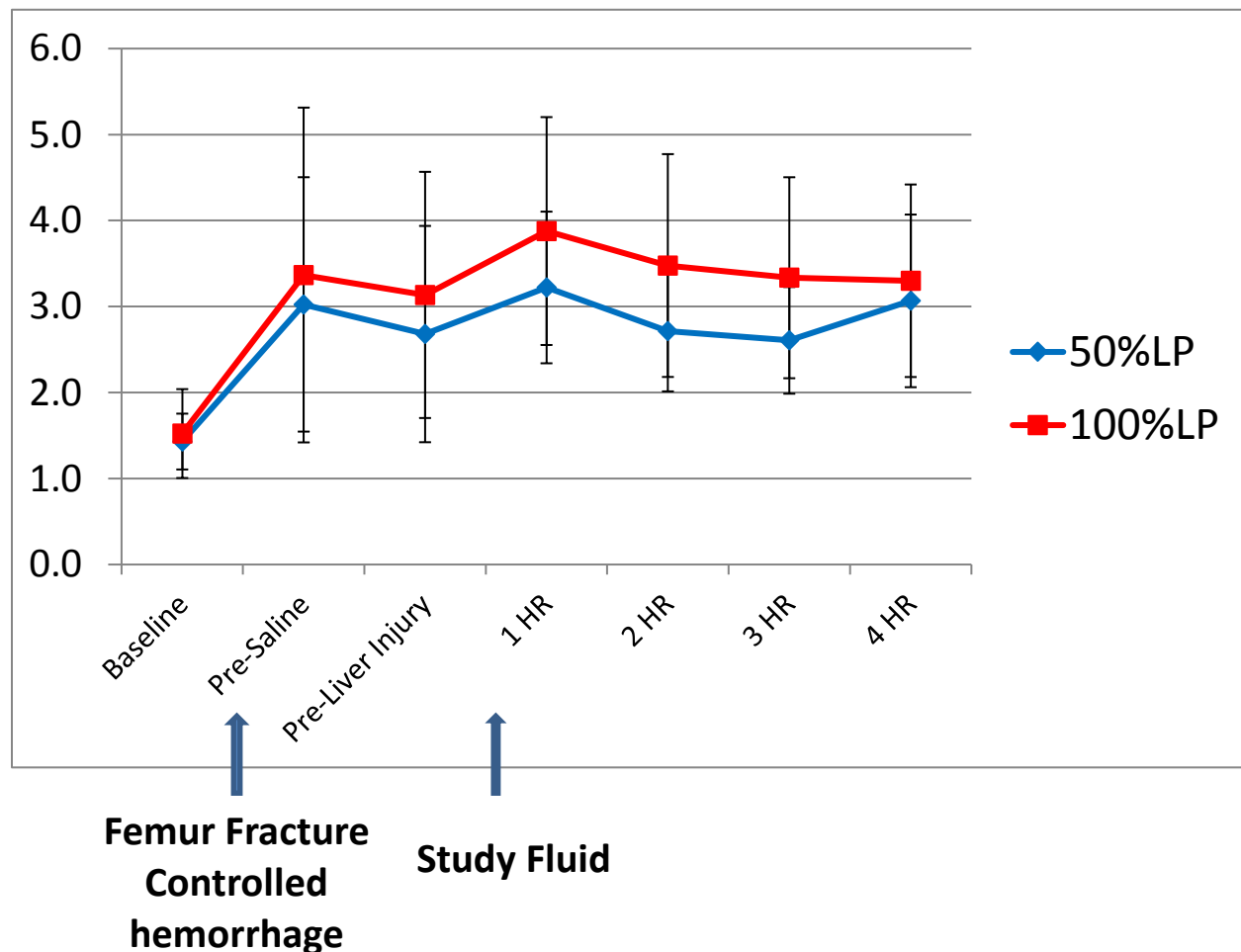


Figure 3. Serum lactate. Data presented as group mean ± SD, $p > 0.05$ at each time point.

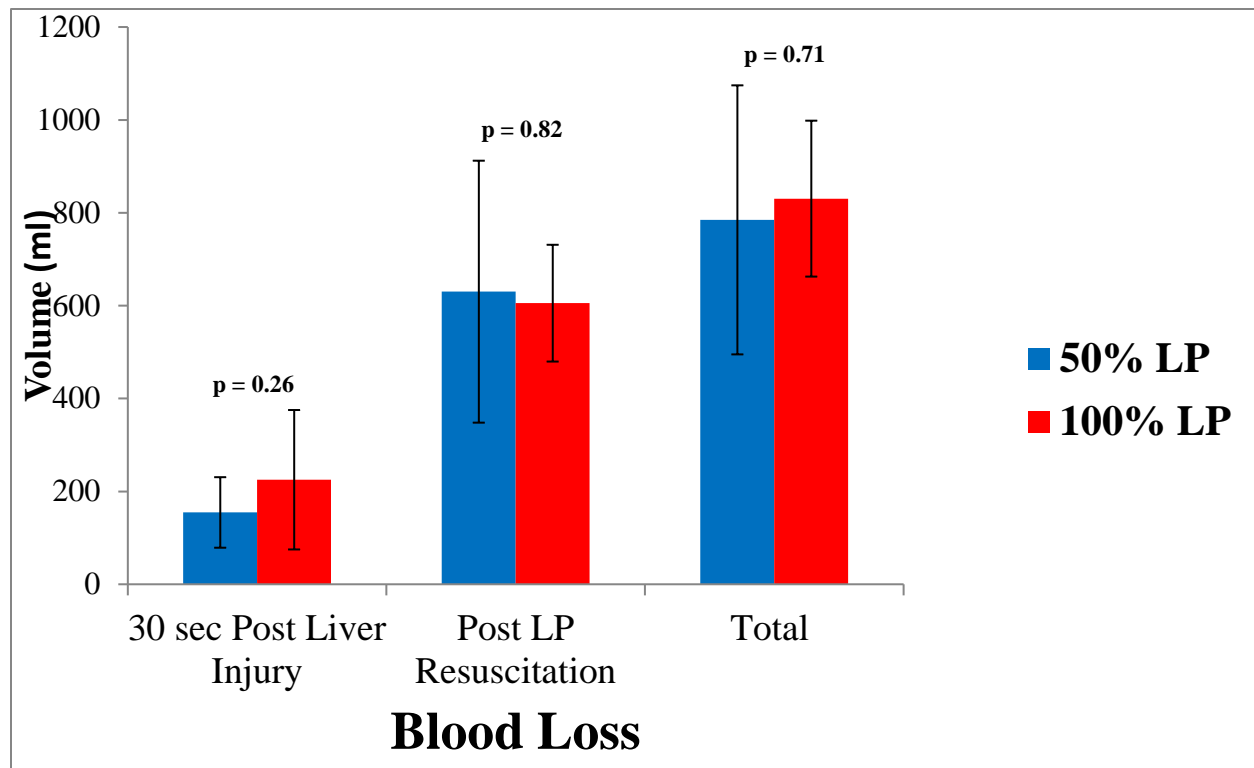


Figure 4. Blood Loss. There was no significant difference in blood loss between study fluid groups (50%LP and 100%LP) for all time points (all $p > 0.05$). All values expressed as mean \pm SD.

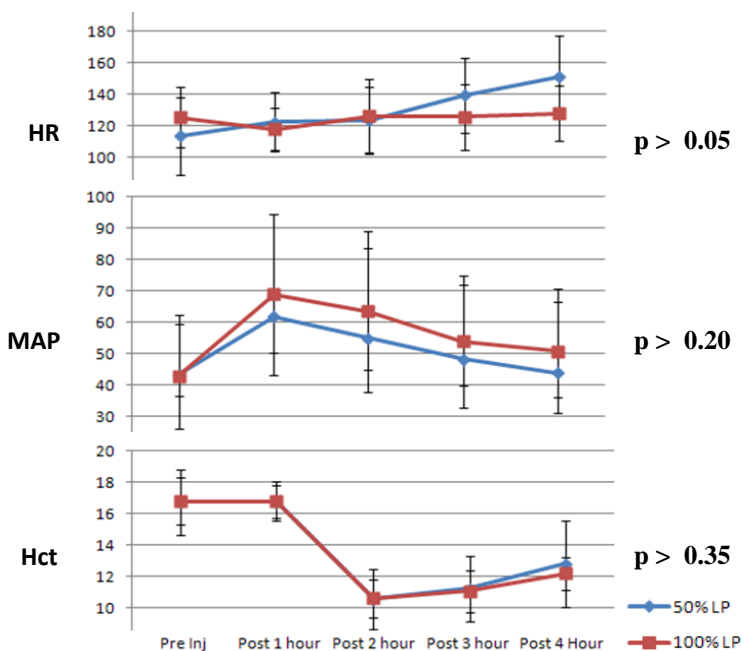


Figure 5. Hemodynamic parameters and Hematocrit (Hct). There was no significant difference in heart rate (HR), mean arterial pressure (MAP), and Hct between study fluid groups (50%LP and 100%LP). All values expressed as mean \pm SD, all $p > 0.05$.

Appendix C – Specific Aim 2 Tables and Figures

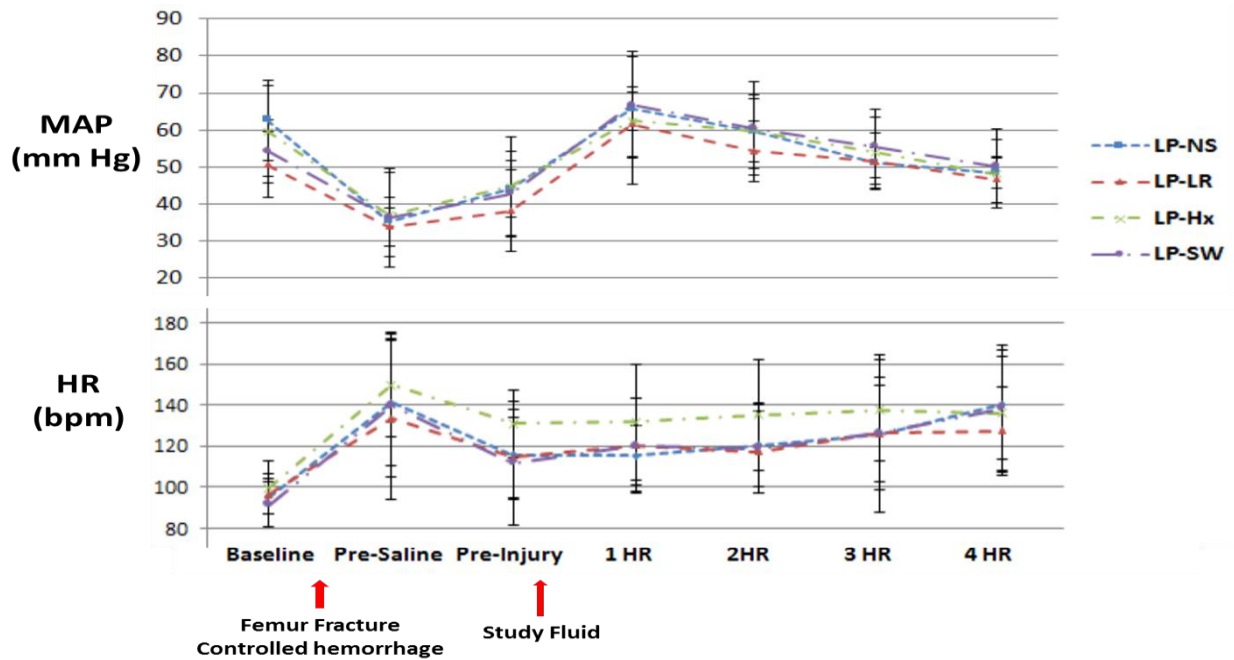


Figure 6. Mean arterial pressure (MAP) and heart rate (HR) of the study fluid groups through the study period. Pre-Saline: before normal 3x normal saline resuscitation, Pre-Injury: before liver injury.

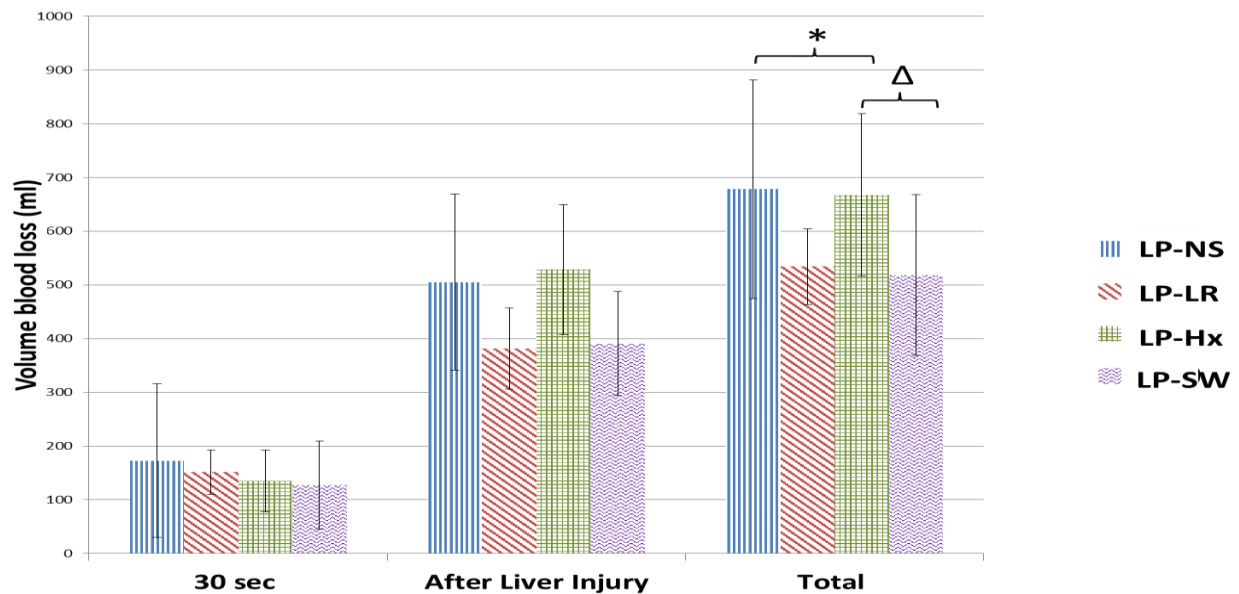


Figure 7. Volume of blood loss following liver injury of study groups. 30 sec: Blood loss measured 30 seconds after uncontrolled hemorrhage following liver injury, After Liver Injury: Blood loss at the end of study, Total: Combined volume of 30 sec and After Liver Injury. *LP-LR < LP-NS and LP-LR < LP-Hx, $p < 0.05$; $^{\Delta}$ LP-SW < LP-Hx, $p < 0.05$.

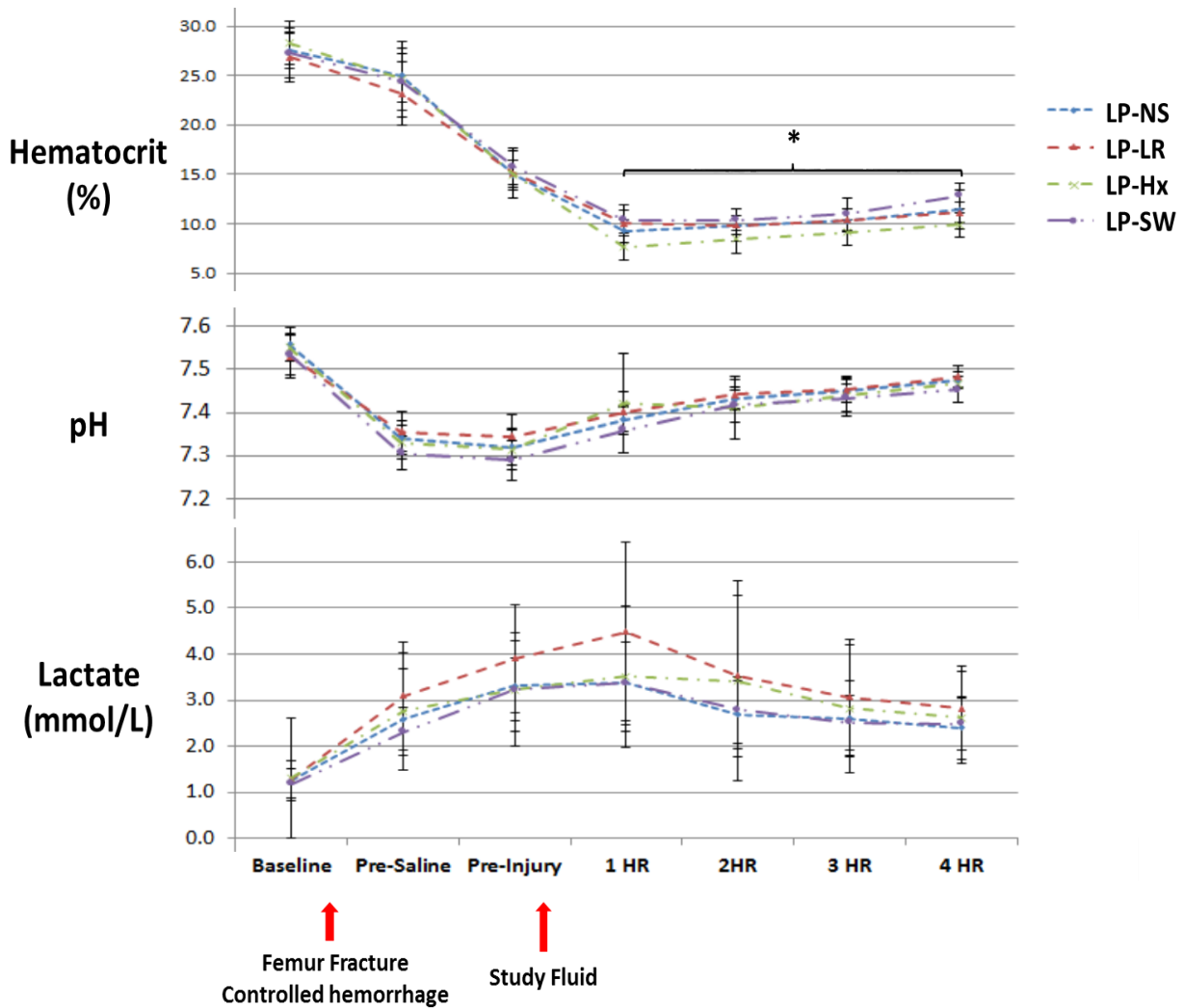


Figure 8. Hematocrit, serum pH, and serum lactate changes of the study fluid groups through the study period. Pre-Saline: before normal 3x normal saline resuscitation, Pre-Injury: before liver injury. *LP-Hx < LP-SW, all time points $p < 0.05$.

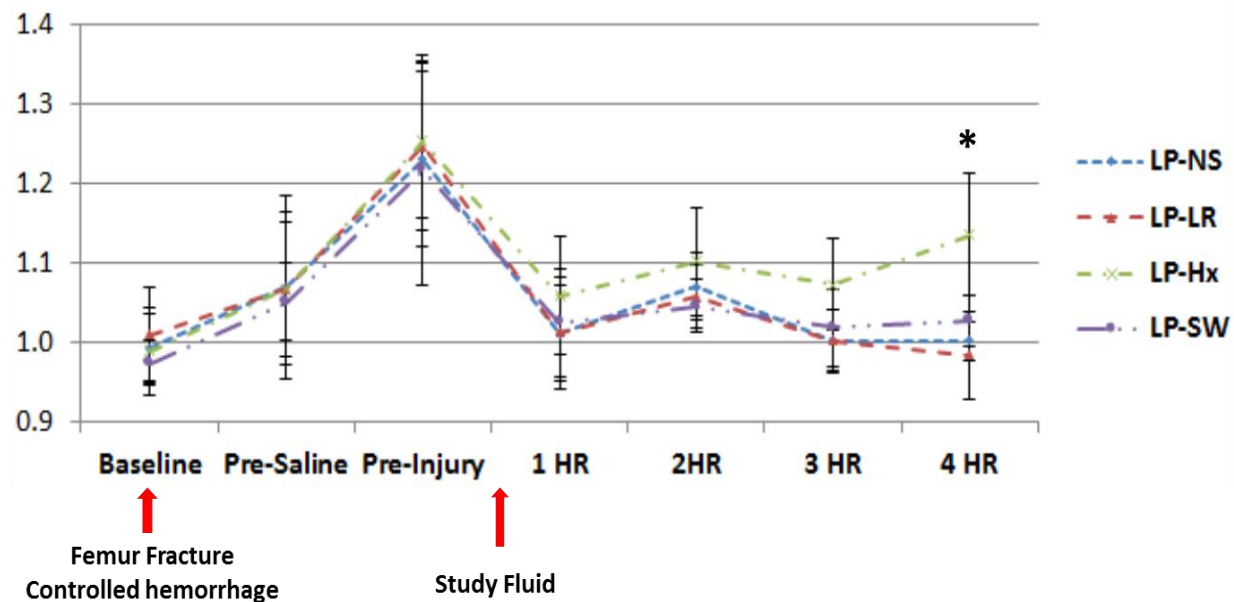


Figure 9. International normalized ratio (INR) for the study fluid groups through the study period. Pre-Saline: before normal 3x normal saline resuscitation, Pre-Injury: before liver injury. *LP-Hx > LP-NS, LP-LR, LP-SW (all $p < 0.05$).

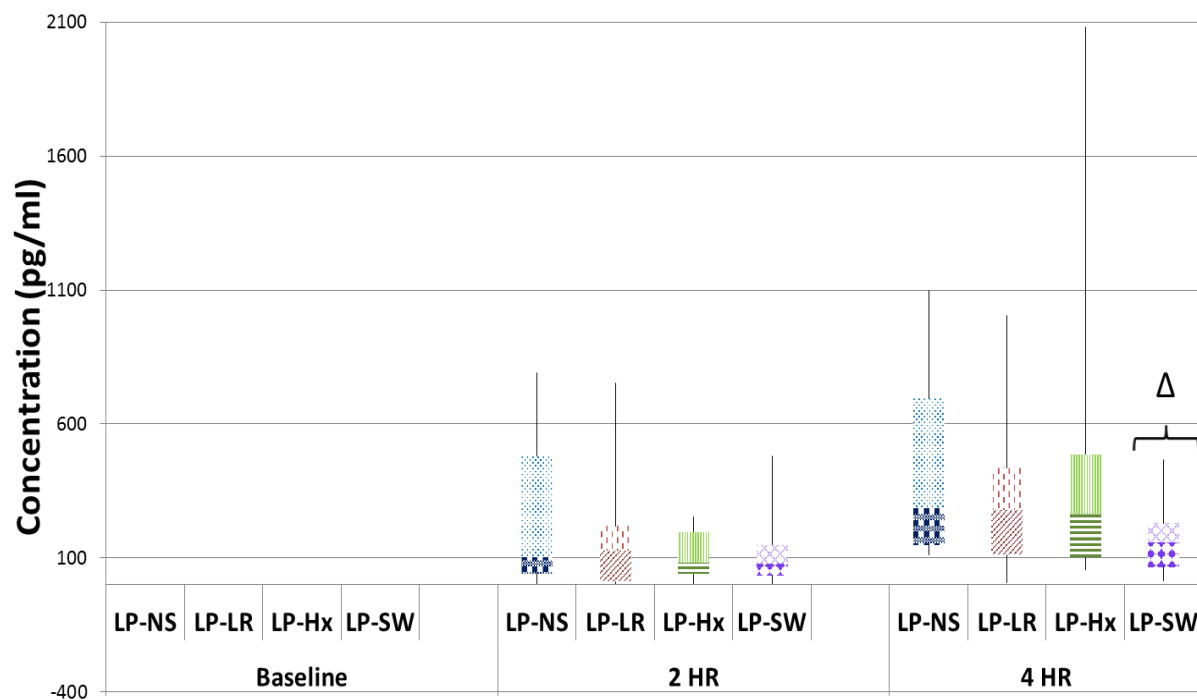


Figure 10. Serum concentration of IL-6 of the study fluid groups through the study period. Pre-Saline: before normal 3x normal saline resuscitation, Pre-Injury: before liver injury. ^Δ LP-SW < LP-NS ($p < 0.05$)

Appendix D – Specific Aim 3 Tables and Figures

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
WEIGHT (kg)		33.0 (33.0, 33.6)	34.3 (31.5, 33.7)	33.2 (31.5, 33.7)	32.0 (31.0, 34.9)	35.0 (34.0, 36.1)
30-SEC BLOOD LOSS (ml)			153 (110, 200)	209 (178, 252)	201 (127, 231)	125 (61, 240)
POST-INJURY BLOOD LOSS (ml)			382 (339, 620)	375 (324, 537)	430 (305, 508)	402 (367, 453)
TOTAL BLOOD LOSS (ml)			585 (480, 690)	589 (524, 787)	624 (546, 667)	555 (488, 629)
LYOPHILIZED PLASMA pH			7.04 (7.02, 7.06)	7.01 (7.00, 7.03)	7.04 (7.02, 7.06)	7.02 (7.00, 7.03)
HEART RATE (bpm)	BL	96 (90, 101)	91 (78, 101)	89 (84, 100)	94 (83, 102)	96 (81, 110)
	1HR	105 (95, 112)	127 (112, 137) *	125 (117, 150) *	118 (102, 144)	124 (115, 147) *
	2HR	108 (104, 125)	133 (115, 145) *	140 (118, 157) *	125 (113, 145)	127 (119, 156)
	3HR	105 (102, 121)	144 (117, 163) *	150 (127, 164) *	134 (120, 157) *	133 (121, 173) *
	4HR	112 (98, 122)	135 (119, 152) *	152 (133, 163) *	142 (127, 158) *	138 (124, 150) *
MEAN ARTERIAL PRESSURE (mmHg)	BL	58.9 (56.6, 65.5)	55.9 (52.6, 61.4)	52.5 (49.9, 58.7)	60.3 (56.2, 64.5)	57.7 (50.2, 62.8)
	1HR	54.3 (46.9, 63.7)	56.1 (52.2, 57.8)	53.7 (50.7, 58.8)	56.4 (52.1, 61.4)	57.2 (52.4, 66.0)
	2HR	52.4 (48.2, 57.8)	53.2 (49.6, 58.1)	51.9 (48.9, 54.6)	54.4 (50.3, 59.1)	54.3 (49.3, 61.8)
	3HR	53.1 (49.1, 56.9)	48.7 (43.8, 53.8)	48.0 (45.1, 50.2)	52.3 (50.0, 54.8)	50.4 (47.0, 55.6)
	4HR	48.6 (46.0, 57.9)	42.8 (39.4, 49.9)	46.3 (42.2, 47.1)	48.3 (46.0, 53.5) #	47.3 (45.1, 51.8)

Table 3: Hemodynamic Variables - Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
BLOOD pH	<i>BL</i>	7.54 (7.50, 7.56)	7.47 (7.44, 7.51) *	7.48 (7.45, 7.51)	7.50 (7.48, 7.53)	7.51 (7.48, 7.54)
	<i>1HR</i>	7.53 (7.48, 7.56)	7.33 (7.29, 7.36) *	7.31 (7.29, 7.37) *	7.35 (7.35, 7.37) *	7.36 (7.31, 7.38) *
	<i>2HR</i>	7.51 (7.48, 7.55)	7.37 (7.33, 7.40) *	7.36 (7.33, 7.39) *	7.39 (7.38, 7.41) *	7.38 (7.36, 7.43) *
	<i>3HR</i>	7.50 (7.47, 7.53)	7.39 (7.34, 7.42) *	7.38 (7.36, 7.40) *	7.42 (7.38, 7.44) *	7.41 (7.37, 7.44) *
	<i>4HR</i>	7.51 (7.46, 7.54)	7.42 (7.34, 7.44) *	7.39 (7.37, 7.40) *	7.43 (7.41, 7.45) *	7.42 (7.40, 7.46) *
CREATININE (mg/dL)	<i>BL</i>	0.8 (0.6, 0.9)	0.7 (0.6, 1.1)	0.8 (0.6, 0.8)	0.8 (0.7, 1.0)	0.9 (0.8, 0.9)
	<i>1HR</i>	1.0 (0.8, 1.1)	0.6 (0.5, 0.9) *	0.7 (0.6, 1.0)	1.1 (0.9, 1.6) #	1.6 (1.5, 1.7) *, #
	<i>2HR</i>	1.0 (0.8, 1.1)	0.7 (0.6, 1.0)	0.8 (0.7, 1.0)	1.1 (0.9, 1.3) #	1.4 (1.0, 1.7) *, #
	<i>3HR</i>	1.1 (0.9, 1.1)	0.8 (0.7, 1.1)	0.8 (0.7, 0.8) *	1.1 (0.9, 1.3) #	1.3 (0.9, 1.4) #
	<i>4HR</i>	1.1 (1.0, 1.1)	0.9 (0.8, 1.2)	0.8 (0.7, 1.0) *	1.2 (1.0, 1.3)	1.2 (1.1, 1.5) *, #
BASE DEFICIT (mmol/L)	<i>BL</i>	-13 (-14, -11)	-8 (-12, -6) *	-10 (-10, -9) *	-10 (-13, -7)	-11 (-14, -11)
	<i>15m</i>	-13 (-15, -12)	5 (3, 7) *	5 (2, 7) *	5 (4, 7) *	5 (2, 6) *
	<i>1HR</i>	-12 (-14, -11)	2 (0, 5) *	1 (-1, 5) *	2 (1, 3) *	2 (0, 3) *
	<i>2HR</i>	-12 (-13, -11)	-1 (2, -2) *	-2 (-3, 0) *	-2 (-2, 2) *	-2 (-3, -1) *
	<i>3HR</i>	-12 (-13, -11)	-3 (-1, -3) *	-3 (-5, -1) *	-3 (-4, -2) *	-4 (-5, -2) *
	<i>4HR</i>	-11 (-11, -10)	-3 (-2, -4) *	-4 (-5, -2) *	-4 (-4, -3) *	-5 (-6, -2) *
LACTATE (mmol/L)	<i>BL</i>	1.6 (1.4, 2.3)	2.0 (1.2, 2.4)	1.6 (1.4, 2.1)	1.4 (1.2, 2.1)	1.5 (1.3, 2.0)
	<i>15m</i>	1.7 (1.3, 2.2)	3.2 (2.8, 3.8) *	3.5 (2.3, 3.8) *	2.7 (2.4, 3.6) *	2.9 (2.2, 3.5) *
	<i>1HR</i>	1.8 (1.1, 1.9)	3.8 (2.8, 4.5) *	3.5 (2.5, 5.0) *	3.0 (2.5, 4.0) *	3.2 (2.7, 3.5) *
	<i>2HR</i>	1.6 (1.0, 1.9)	3.2 (2.7, 3.8) *	2.8 (2.2, 4.4) *	2.7 (2.1, 3.4) *	2.7 (2.2, 3.4) *
	<i>3HR</i>	1.4 (1.2, 2.1)	2.7 (2.5, 3.1) *	2.4 (2.1, 3.6) *	2.5 (1.9, 3.2) *	2.4 (1.8, 3.1) *
	<i>4HR</i>	1.4 (1.2, 2.1)	2.5 (2.0, 3.9) *	2.1 (1.8, 3.5) *	2.0 (1.7, 2.9)	2.2 (1.7, 2.8) *

Table 4: Blood Chemistry Values - Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as p < 0.05 versus OCS (*) and p < 0.05 versus HCL (#).

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
WHITE BLOOD CELLS (x1000)	<i>BL</i>	17.0 (15.4, 18.3)	17.2 (16.0, 20.0)	15.7 (12.0, 23.2)	16.7 (14.1, 17.9)	17.0 (14.6, 19.8)
	<i>1HR</i>	18.3 (10.7, 25.0)	6.0 (4.0, 6.1) *	4.9 (4.3, 6.7) *	5.1 (4.5, 5.5) *	5.1 (3.9, 6.9) *
	<i>2HR</i>	18.1 (11.2, 27.1)	7.3 (5.0, 9.0) *	7.7 (7.2, 8.6) *	7.1 (6.1, 10.0) *	8.5 (5.3, 10.2) *
	<i>3HR</i>	17.8 (13.1, 27.6)	9.4 (8.4, 12.2) *	11.1 (9.9, 12.8) *	12.3 (11.3, 14.2)	11.0 (8.2, 13.1) *
	<i>4HR</i>	18.6 (13.9, 25.6)	12.7 (10.8, 15.6) *	14.6 (13.1, 15.2)	15.1 (13.5, 15.8)	14.8 (10.9, 16.6)
HEMATOCRIT (%)	<i>BL</i>	26.5 (23.8, 27.8)	25.0 (24.6, 27.6)	27.0 (24.8, 29.3)	28.0 (26.8, 29.3)	27.0 (26.0, 29.9)
	<i>1HR</i>	27.8 (26.0, 29.4)	11.8 (9.9, 12.6) *	12.8 (10.5, 13.1) *	10.8 (9.9, 12.3) *	11.8 (10.5, 12.6) *
	<i>2HR</i>	28.5 (27.5, 29.3)	11.3 (10.0, 12.0) *	12.0 (10.8, 13.0) *	10.1 (9.9, 10.9) *	11.0 (10.0, 11.6) *
	<i>3HR</i>	28.0 (27.1, 29.3)	10.8 (9.9, 12.5) *	12.0 (11.4, 12.6) *	10.0 (10.0, 11.9) *	11.8 (10.8, 12.5) *
	<i>4HR</i>	28.8 (28.0, 29.3)	12.3 (10.8, 13.0) *	12.3 (10.8, 14.0) *	11.3 (10.0, 12.3) *	11.8 (11.3, 13.1) *
PLATELETS (x1000/mm3)	<i>BL</i>	187 (149, 251)	243 (201, 338)	172 (118, 245)	270 (204, 367)	177 (131, 256)
	<i>1HR</i>	208 (131, 256)	197 (180, 257)	185 (165, 231)	238 (184, 264)	208 (179, 247)
	<i>2HR</i>	186 (135, 229)	209 (198, 266)	186 (157, 246)	226 (181, 251)	200 (162, 222)
	<i>3HR</i>	166 (156, 240)	211 (192, 258)	181 (163, 239)	216 (174, 263)	194 (158, 209)
	<i>4HR</i>	187 (136, 219)	188 (172, 237)	175 (143, 228)	213 (161, 251)	175 (148, 187)
ACTIVATED CLOTING TIME (secs)	<i>BL</i>	113 (104, 126)	129 (116, 138)	120 (115, 128)	126 (114, 136)	127 (112, 135)
	<i>1HR</i>	124 (118, 129)	164 (148, 179) *	161 (150, 183) *	157 (140, 167) *	169 (151, 189) *
	<i>2HR</i>	116 (109, 123)	155 (131, 168) *	154 (136, 166) *	137 (130, 150) *	147 (139, 166) *
	<i>3HR</i>	113 (111, 116)	136 (130, 164) *	144 (128, 154) *	135 (112, 155)	138 (125, 164) *
	<i>4HR</i>	112 (105, 126)	140 (131, 151) *	138 (129, 147) *	133 (121, 141) *	136 (122, 146) *

Table 5: Complete Blood Count & Activated Clotting Time - Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).

		OCS	HCL	LOW AA	MEDIUM AA	HIGH AA
R (minutes)	BL	9.0 (7.7, 10.3)	8.7 (7.9, 10.7)	9.5 (8.2, 13.2)	10.5 (9.3, 11.4)	8.5 (7.0, 13.0)
	1HR	7.6 (6.4, 8.3)	7.7 (7.0, 9.9)	9.5 (8.8, 11.7) *, #	10.0 (8.1, 11.6) *, #	10.2 (7.8, 11.1) *
	2HR	7.9 (5.2, 8.6)	8.0 (7.4, 9.4)	7.7 (7.2, 9.7)	8.8 (6.2, 9.3)	9.4 (6.2, 10.7)
	3HR	6.8 (5.6, 7.2)	7.3 (6.0, 9.0)	8.6 (6.7, 9.9)	8.0 (6.7, 9.6)	7.9 (6.4, 10.2)
	4HR	6.0 (4.6, 7.6)	6.1 (5.4, 7.5)	8.3 (7.0, 9.2) *, #	7.1 (6.1, 8.4)	8.0 (6.0, 10.1)
K (minutes)	BL	2.0 (1.7, 2.1)	1.7 (1.4, 1.9)	1.8 (1.4, 2.8)	1.8 (1.7, 2.1)	1.7 (1.3, 2.1)
	1HR	1.6 (1.4, 1.7)	2.4 (1.9, 3.6) *	3.5 (2.4, 5.4) *	3.2 (2.6, 3.5) *	2.9 (2.3, 4.8) *
	2HR	1.5 (1.2, 1.6)	2.5 (1.8, 3.3) *	2.2 (1.7, 3.1) *	2.3 (1.8, 2.6) *	2.2 (1.7, 3.5) *
	3HR	1.4 (1.2, 1.5)	2.3 (1.5, 2.9) *	2.2 (1.6, 3.3) *	1.9 (1.8, 2.5) *	1.9 (1.4, 3.0) *
	4HR	1.3 (1.1, 1.5)	1.8 (1.3, 2.3)	2.1 (1.5, 2.9) *	1.6 (1.5, 2.2) *	1.8 (1.3, 3.4) *
α (degrees)	BL	65.6 (36.2, 71.2)	69.5 (66.3, 72.8)	64.7 (61.1, 67.5)	67.8 (65.5, 69.8)	70.2 (64.0, 74.1)
	1HR	70.0 (67.7, 72.7)	62.4 (59.5, 68.2) *	58.8 (50.5, 64.2) *	59.0 (56.3, 62.1) *	59.3 (46.9, 65.0) *
	2HR	71.5 (69.0, 74.6)	63.2 (58.5, 70.1) *	64.5 (60.6, 69.3) *	64.1 (61.2, 68.3) *	65.0 (56.6, 70.2) *
	3HR	72.2 (70.4, 74.5)	65.0 (60.6, 72.9)	64.2 (59.3, 70.8) *	66.6 (62.3, 68.7) *	67.6 (59.8, 72.4)
	4HR	72.2 (70.9, 76.3)	70.1 (66.2, 73.7)	66.2 (62.7, 71.4) *	69.6 (65.5, 71.5)	67.0 (57.1, 73.6)
CI (AU)	BL	0.6 (-0.8, 1.4)	1.4 (-0.4, 2.1)	0.3 (-3.1, 1.7)	-0.3 (-1.4, 1.0)	1.6 (-2.1, 2.8)
	1HR	2.0 (0.8, 3.3)	0.4 (-1.2, 1.3) *	-1.2 (-3.4, -0.2) *, #	-1.4 (-2.7, 0.2) *	-1.1 (-3.8, 0.8) *
	2HR	1.8 (1.0, 3.9)	0.0 (-1.0, 2.1)	1.0 (-0.9, 1.8)	0.3 (-0.7, 2.3) *	-0.2 (-2.6, 2.4)
	3HR	2.8 (2.2, 3.8)	0.7 (-0.2, 2.9)	0.5 (-0.9, 2.2)	1.1 (-0.7, 2.1) *	1.0 (-1.4, 2.7)
	4HR	2.9 (2.2, 4.6)	2.8 (1.5, 3.6)	0.8 (0.0, 2.2) #	2.0 (0.8, 2.8)	1.1 (-1.8, 3.1)

Table 6: TEG Variables - Data presented as median (IQR). Columns represent operative control sham (OCS), hydrochloric acid (HCL) control and low, medium and high ascorbic acid (AA) concentrations. Significance defined as $p < 0.05$ versus OCS (*) and $p < 0.05$ versus HCL (#).